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**The Evolution of Energy-Transducing Systems. Studies with an Extremely  
Halophilic Archaeobacterium.**

Final Report

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Principal Investigator: Helga Stan-Lotter

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Attached:

Copies of all papers published or in press

1)The NASA Technical Officer for this grant was Dr. L.I.Hochstein, formerly at NASA Ames Research Center, Moffett Field, CA 94035, present address: University of Santa Cruz, Department of Chemistry and Biochemistry, Santa Cruz CA 95064.

2)Abbreviations: DCCD, dicyclohexylcarbodiimide; NEM, N-ethylmaleimide.

## Introduction:

The F-type ATPases are found in remarkably similar versions in the energy-transducing membranes of bacteria, chloroplasts and mitochondria (1). Thus, it is likely that they have originated early in the evolution of life, which is consistent with their function as key enzymes of cellular metabolism. The archaea (formerly called archaebacteria) are a group of microorganisms which, as shown by molecular sequencing and biochemical data, have diverged early from the main line of prokaryotic evolution (2). From studies of members of all three major groups of archaea - the halophiles, methanogens and thermoacidophiles - it emerged that they possess a membrane ATPase, which differs from the F-ATPases. The goal of this project was a comparison of the ATPase from the halophilic archaebacterium *Halobacterium saccharovorum* with the well-characterized F-type ATPases on the molecular level. The results were expected to allow a decision about the nature of archaebacterial ATPases, their classification as one of the known or, alternatively, novel enzyme complex, and possibly a deduction of events during the early evolution of energy-transducing systems.

## Summary of research results:

### ATPase structure and mechanism

Labeling studies in Dr. Hochstein's laboratory had indicated a preferential incorporation of an inhibitor of F-type ATPases, DCCD<sup>2</sup>), into the second largest subunit (subunit II) of the ATPase from *H. saccharovorum* (3), under conditions, which were similar to those for DCCD-labeling of the  $\beta$  subunit of the F-ATPase of bacteria and mitochondria. Thus, the halobacterial ATPase was thought to be an F-type ATPase. However, nucleic acid sequence information from archaeal and eukaryotic ATPases suggested extensive homologies between the DNA sequences of the major ATPase subunits from archaea with the vacuolar ATPases (4-6), which are found in the endomembrane system of eukaryotes and are, like the F-ATPases, multisubunit complexes (7). The catalytic site is located in the largest subunit (subunit A). Comparisons of the halobacterial ATPase with vacuolar ATPases were made with the collaboration of Drs. B. and E. Bowman at the University of Santa Cruz. The experiments yielded rather clear-cut results: crossreaction of subunit I of the halobacterial ATPase with an antiserum raised against subunit A of the vacuolar ATPase from *Neurospora crassa*; recognition of only one peptide of the size of subunit I in halobacterial membranes by the same antiserum; inhibition of the halobacterial ATPase by NEM<sup>2</sup>), as shown for V-ATPases; demonstration of the incorporation of NEM into subunit I at a cysteinyl residue; reduction of that incorporation by nucleotides and concomitant reduction of inhibition. In addition, it could be shown that the antiserum against subunit A recognized a polypeptide of similar mobility as subunit I in various halophilic bacterial isolates. An extension of this work to the ATPase of another archaeon, *Sulfolobus solfataricus*, which had been studied in the laboratory of Dr. Hochstein, suggested the similarity of that enzyme to the vacuolar ATPases as well. The conclusions drawn from these results were the existence of similarities between archaeal and vacuolar ATPases in structure and mechanism, which have been published (Papers

1991, 1992; abstracts 1989-1994, 1996, 1997). Work still in progress, which is expected to be completed, are the determination of the sequence of the NEM-binding site and the mechanism of inhibition by DCCD (abstracts 1994, 1996).

## **Evolution of energy-transducing enzymes**

From sequence and structural homologies it is evident, that V- and F-ATPases descended from a common ancestor; in addition, the two major subunits of each of the enzyme complexes show homologous regions, which suggests gene duplication events. The distribution of ATPase types among prokaryotes (archaea and bacteria) is of ongoing interest; the initial assumption, that prokaryotic V-ATPases are associated exclusively with archaea, was not tenable, since V-ATPases had been detected in bacteria such as *Thermus* and *Enterococcus* species. Our studies with antibodies against the halobacterial ATPase lead to the discovery, that V- and F-ATPases occur in closely related strains of the same genus (paper submitted; abstracts 1996, 1997).

From salt sediments of the Permian or Triassic period, viable strains of halobacteria were isolated by us and other workers. Although the age of the deposits - between 190 to 250 million years before present - is "short" in geological and evolutionary terms, it is intriguing to compare the ATPases from such organisms with those of surface bacteria. If the halobacteria from salt are the remnants of populations which inhabited the original brines, evolution has stopped for them since the time of deposition. Comparisons of enzymes on the sequence level might provide information about the molecular clock of halobacteria, perhaps also about gene transfer in early environments. We isolated a novel halococcus from Austrian salt sediments, which was deposited in major culture collections. Results obtained so far with Western blots suggested the presence of a V-ATPase. The organism may be able to assimilate carbon by a novel mechanism (Papers 1992, 1993, 1994; abstracts 1992, 1994, 1996, 1997).

## **Novel methods**

Although halobacteria have been studied in detail for over 30 years and the recognition of this group as members of the archaea dates back to 1977, comparatively few studies deal with their proteins on a molecular level. This reflects methodological problems inherent in the requirement of many halobacterial enzymes and other proteins for high concentrations of salt, often in the range of 4 M NaCl. At such high ionic strength, few detergents are soluble, and existing methods had to be modified extensively. Methods, which were developed in the course of this work, include gel electrophoresis of whole cell proteins of halobacteria and isoelectric focussing. Both can serve as rapid tests in the identification of strains; isoelectric focussing has the additional advantage of separating the extremely acidic proteins of typical halobacteria. Another method which we developed, is Western blotting from dried and stained gels; this allows successful identification of small amounts of proteins on stored gels with newly available highly sensitive reagents, e.g. chemiluminescent labels (Papers 1989, 1997; abstracts 1989, 1992).

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7. Forgacs, M. (1989) Structure and Function of vacuolar class of ATP-driven proton pumps. *Physiol. Rev.* 69, 765-796.

## Publications resulting from NCC2-578

### Papers

- Hochstein, L.I., Emrich, E. and Stan-Lotter, H. Inhibition of the vacuolar-like ATPase from *Halobacterium saccharovorum* by p-chloromercuriphenylsulfonate: evidence for the presence of more than one essential sulfhydryl group. (in preparation)
- Radax, C., Sigurdsson, O., Hreggvidsson, G.O., Aichinger, N., Gruber, C., Kristjansson, J.K. and Stan-Lotter, H. F- and V-ATPases in the genus *Thermus* and related species (submitted to *System. and Appl. Microbiol.*)
- Gruber, C. and Stan-Lotter, H. (1997) Western blot of stained proteins from dried polyacrylamide gels. *Anal. Biochem.* (in press)
- Denner, E.B.M., McGenity, T.J., Busse, H.-J., Wanner, G., Grant, W.D. and Stan-Lotter, H. (1994) *Halococcus salifodinae* sp. nov., an Archaeal isolate from an Austrian salt mine. *Internat. J. System. Bacteriol.* 44, 774-780.
- Stan-Lotter, H., Sulzner, M., Egelseer, E., Norton, C.F. and Hochstein, L.I. (1993) Comparison of membrane ATPases from extreme halophiles isolated from ancient salt deposits. *Origins of Life and Evolution of the Biosphere* 23, 53-64
- Stan-Lotter, H. and Denner, E. (1992). On the isolation of halophilic microorganisms from

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Stan-Lotter, H., Lang, F.J. Jr. and Hochstein, L.I. (1989) Electrophoresis and isoelectric focusing of whole cell and membrane proteins from the extremely halophilic archaeobacteria. Appl.Theor. Electrophor.1,147-153.

## Abstracts

Legat, A., Glaser, K. and Stan-Lotter, H. (1997) Carbon assimilation by halophilic archaea. Gem. Jahrestagg. ÖBG u.ÖGGGT, Wien 1997.

Aichinger, N., Radax, C., Sigurdsson, O. and Stan-Lotter, H. (1997) Distribution of F- and V-ATPases in the genus *Thermus*. Gem. Jahrestagg. ÖBG u.ÖGGGT, Wien 1997.

Stan-Lotter, H. and Glaser, K. (1996) Carbon assimilation by *Halococcus salifodinae*, an archaeobacterial isolate from Permian salt deposits. Abstr. c4.4, p. 54, 8th ISSOL Meeting, Orleans, France (talk)

Stan-Lotter, H., Hochstein, L.I. and Gruber, C. (1996) Immunological approaches to the evolution of halobacterial and eubacterial membrane ATPases. Abstr.p.86, First Int. Congr. Extremophiles, Estoril, Portugal (talk)

Radax, C. and Stan-Lotter, H. (1996) A Proteolipid from Halobacteria and Natronobacteria with similarity to subunit c of F-type ATP synthases. Abstr.p.196, First Int. Congr. Extremophiles, Estoril, Portugal

Glaser, K. and Stan-Lotter, H. (1996) Carbon assimilation by *Halococcus salifodinae*, an isolate from Permian salt deposits. Biospektrum Sonderausgabe 1996, Abstr. PE 159, p.126, VAAM Frühjahrstagg. Bayreuth

Sigurdsson, O. and Stan-Lotter, H. (1996) Presence of V- and F-type ATPases in the membranes from *Thermus scotoductus*. Biospektrum Sonderausgabe 1996, Abstr. PE 001, p.100, VAAM Frühjahrstagg. Bayreuth

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similar to subunit c of F-type ATP synthase. Biospektrum Sonderausgabe 1996, Abstr. PE 160, p.126, VAAM Frühjahrstag. Bayreuth

Radax, C., Stan-Lotter, H. and Hochstein, L.I. (1994) A proteolipid from *Halobacterium saccharovorum* with similarity to subunit c of *Escherichia coli*. Abstr. I-86, ASM Meeting, Las Vegas 1994.

Hochstein, L.I., Stan-Lotter, H. and Emrich, E. (1994) Inhibition of the vacuolar-like ATPase from *Halobacterium saccharovorum* by thiol reagents : evidence for different functional thiols. Abstr. K-97 ASM Meeting, Las Vegas 1994.

Stan-Lotter, H., Denner, E., McGenity, T.J., Busse, H.J., Grant, W.D. and Wanner, G. (1994) A novel archaeal isolate from an Austrian salt mine. Abstr. P 152, Bioengineering 3/94, pg. 43

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Stan-Lotter, H., and Hochstein, L.I. (1992). Immunological Approaches to the evolution of halobacterial ATPases. Abstr.22, ASM Conf.Halophilic Bacteria,Williamsburg VA.

Denner, E., Grant, W.D. and Stan-Lotter, H. (1992) A halophilic coccoidal isolate from salt sediments. Abstr.15, ASM Conf.Halophilic Bacteria,Williamsburg VA.

Denner, E., Busse, H.J. and Stan-Lotter, H. (1992) A novel archaeal isolate from Austrian salt sediments. Gem.Jahrestagg.ÖBG u.ÖGGGT,Innsbruck 1992.

Stan-Lotter, H., Lang, F.J., Richter, M., Neuninger, C. and Hochstein, L.I. (1992) Rapid identification of halobacterial species by protein gel electrophoresis. Abstr. I-34, ASM Meeting, New Orleans

Stan-Lotter, H. (1991) Comparison of proteins from halophilic archaeobacteria and microbial isolates from ancient salt deposits. Abstr. Eur.Geophys.Soc. XVI Ass. Wiesbaden, PS 11 (talk)

Sulzner, M., Stan-Lotter, H., and Hochstein, L.I. (1991). Nucleotide protectable labeling of sulphhydryl groups in subunit I of the ATPase from *Halobacterium saccharovorum*. Abstr.K-73, ASM Meeting,Dallas.

Sulzner, M., Egelseer, E. and Stan-Lotter, H. (1991) Analysis of archaeobacterial and eubacterial ATPases by radiation inactivation and immunoreactions. Abstr.P 16 VAAM Meeting, Freiburg. Bioforum 14, 39.

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*saccharovorum* is a chimeric enzyme. Abstr. Int. Conf. Archaeobacteria, Osaka, Nov.1990, pg.13.

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Sulzner, M. and Stan-Lotter, H. (1989) Evolution of membrane ATPases. Studies with extremely halophilic archaeobacteria. Gem.Jahrestagg.ÖBG u.ÖGGGT,Wien 1989, B 34.

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### **Other related activities:**

#### **Public lecture**

May 3, 1995: University of Salzburg, Austria,

Title: Where do microorganisms come from ? (in German). This talk received coverage by all major Austrian newspapers.

#### **Invited talks:**

May 8, 1996: Soroptimists Germany, Ravensburg,

Title: Microorganisms and the search for extraterrestrial life (in German)

March 10, 1997: Ambassador's Club, Stuttgart, Germany,

Title: Microorganisms and the search for life in outer space (in German)